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13. ABSTRACT *(Maximum 200 words)*

Metastatic and primary tumor growth are both dependent on neovascularization. One can then predict that restrictions in the vascular supply to tumors could impinge on the expansion and uncontrolled growth of tumor cells. Studies presented in this proposal aim to evaluate the effect of an inhibitor of angiogenesis, thrombospondin-1 (TSP-1), in the growth of mammary tumors. The advantage of TSP-1 over other vascular inhibitors is that this protein is a natural angiogenic suppressor. TSP-1 is produced by the mammary gland during post-lactation and in fact contributes to the regression of capillaries during mammary gland involution. Therefore, we hypothesized that if effective in the regression of tumors, this molecule might not induce secondary or undesirable side effects.

We have just completed two years of studies in this proposal. Our findings are encouraging; specifically, we were able to demonstrate in the lack of TSP-1 (utilizing TSP-1 null animals), neovascularization is significantly higher than in the presence of endogenous TSP-1. Furthermore, increase in the endogenous pool of TSP-1 induces apoptosis in capillary endothelial cells. The overall effect is a reduction in the vascular supply to the mammary gland and to the highly vascularized tumors. Experiments within the next two years of this proposal will aim to understand the molecular mechanism by which TSP-1 mediates endothelial cell apoptosis.

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INTRODUCTION

1. Original Abstract (from grant proposal)

Growth and metastasis of breast cancer is directly dependent on neovascularization. By understanding the mechanisms that control the neovascular response, it may be possible to design therapeutic strategies to selectively prevent or halt pathological growth of vessels and consequently restrain the progression of cancer cells. Despite its general biological significance and pathological relevance, relatively little is known about inhibitors of blood vessel formation. Thrombospondin-1 (TSP1), a glycoprotein originally described as a major component of platelet α-granules, has recently been identified as a negative regulator of angiogenesis. Its relevance for the suppression of vascular growth in tumors has yet to be investigated.

The present proposal was designed to address the role of TSP1 in the neovascularization of mammary tumors. Initially, kinetics of vascular development will be examined in mammary tumors of TSP1-deficient mice and in tumors of control animals. A second set of experiments will focus on the effect of TSP1 in normal and tumor-derived endothelial cells at the cellular and at the molecular level. Specifically, we will investigate the proliferation, invasion, and chemotaxis of normal and tumor-derived endothelial cells in a model of angiogenesis *in vitro*. A final facet of this proposal is directed to investigate the modulation of TSP1 receptors as both populations of cells organize into cords and tubes *in vitro* and to identify the receptor(s) responsible for the generation of signals ultimately responsible for the regulation of endothelial cell behavior in breast cancer.

2. Relevance of the present work to Breast Cancer

Partial reduction or suppression of tumorigenicity can be accomplished at multiple levels: by direct cell cycle regulation, targeted cellular ablation, control of signal transduction, and/or inhibition of angiogenesis (reviewed in refs. 1-6). Several investigators have implicated tumor suppressor genes in cell cycle regulation or signal transduction pathways and considerable effort is being made to identify the critical points in cell transformation that might be sensitive to pharmacological control. A parallel line of investigation has focused on understanding the **regulation of vascular growth in cancer.** It is recognized that an increase in the vascular supply plays a central role in tumor progression and metastasis (5-9). In fact in breast cancer, angiogenesis has been acknowledged as a significant indicator of tumor progression that is independent of axillary lymph node status (9-11). Although of recognized relevance, therapeutic approaches have generally excluded treatment of breast cancer by target ablation of neovascular growth; mostly because to date, angiogenic inhibitors have proven either too generally toxic or not selective to particular vascular beds. This proposal offers a <u>new perspective</u> into the concept of vascular inhibitors by focusing our attention on a natural angiogenic inhibitor present in normal mammary glands: the glycoprotein thrombospondin-1 (TSP1). According to our preliminary data, TSP1 seems to be suppressed during pathological neovascularization of breast tumors, therefore it is our premise that an exogenous supply of TSP1 should be effective and non-toxic. In addition, TSP1 seems to be specific to steroid-dependent tissues, which could potentially offer selectivity in the inhibition of mammary vessels.

3. Backgound and previous work done by the applicant

TSP1 has been identified as an inhibitor of angiogenesis both *in vivo* and *in vitro* (12-14). Interestingly, TSP1 has also been acknowledged as a tumor-suppressor gene in human-hamster cell hybrids and has been implicated in the inhibition of the tumorigenic ability of MCF-7 breast cancer cells (15). In addition, we recently found that TSP1 mRNA is regulated by steroids at the transcriptional level, an interesting finding considering the implication of steroids in the

development of certain mammary tumors (16) Examination of the vasculature of human mammary glands reveals consistent expression of TSP in the capillaries of normal tissue; its expression in tumors, however is not as steady. TSP1 was indeed rarely present in capillaries of the human tumors examined. Although the casual correlation between lack of TSP1 and rapid growth of tumors is attractive, whether its absence is associated with the rampant growth and capillary progression of those tumors and whether administration of TSP1 could reverse the abnormal growth of capillaries in mammary tumors remains to be tested.

Thrombospondin in the mammary gland and other steroid-dependent tissues.

High levels of TSP1 have been reported in milk, other breast secretions, and in some types of mammary cystic fluids (17). With the exception of bone and circulating platelets, the mammary gland represents one of the few adult tissues with high concentrations of TSP. Interestingly, a study on the kinetics of TSP in human milk has shown temporal variations which could be due to hormonal regulation. TSP was detected in the initial "aqueous phase" of milk secretion, its levels subsequently fell during the transition to mature milk (18). We have studied the expression of TSP1 in 32 hysterectomy cases. Our results showed stage-dependent regulation of this gene in the human endometrium (Table 1 and appendix - manuscript). Specifically we identified TSP1 protein in capillaries of the functional endometrium and this expression correlated with the end of the endometrial cycle (secretory phase). Tissues from the early proliferative phase showed no immunoreactivity. Identification of vessels with an anti-CD 34 antibody in serial sections demonstrated that only a subset of capillaries was reactive with anti-TSP-1 antibodies. Moreover, immunostaining with PCNA (proliferating cell nuclear antigen) IgG indicated that the presence of TSP-1 protein did not, in all instances, correlate with proliferating endothelial cells. Since neovascularization is also regulated by a series of inhibitory signals, we propose that TSP1 is required at later stages of the endometrial cycle to inhibit vessel formation or to stabilize newly-formed capillaries. We have also performed *in situ* hybridization on similar sections to localize TSP1 transcripts. Abundant expression of TSP1 mRNA was identified in the secretory phase, in contrast to the low levels detected in the proliferative phase. TSP1 mRNA was observed not only in endothelial cells, but also in stromal cells of the human endometrium. It was interesting that no protein was detected in stromal cells by immunocytochemistry. These observations suggest that, although stromal cells might secrete high levels of TSP1, the protein is accumulated only in the basement membranes of vessels, where it supposedly exerts its anti-angiogenic effect. Therefore, it is our presumption that TSP1 might act in a paracrine or autocrine manner to regulate vessel growth. Indeed, this hypothesis could be extended to other systems, since significant levels of TSP1 protein are also secreted by vascular smooth muscle cells (19). In the secretory phase, the distinction between the stratum functionalis and the stratum basalis with regard to TSP1 mRNA expression was striking. Whereas high levels were observed in the stratum functionalis, only background levels, equivalent to the intensity observed during the proliferative phase, were seen in the stratum basalis. These findings suggested to us that the TSP1 gene might be, at some level, regulated by steroids.

We have examined the regulation of TSP1 mRNA in cultured cells exposed to progesterone. Steady-state levels of TSP1 mRNA were elevated 4.5 fold in human stromal endometrial cells at 6h after treatment with progesterone. This effect was dose-dependent and was mediated at the transcriptional level, as shown by nuclear run-on experiments. In this context, it is interesting that analysis of the mouse and human TSP1 promoters reveal the presence of a consensus sequence (AGTCCT) (20) that has been reported to interact with the glucocorticoid receptor (21).

In the mammary gland, we have detected TSP1 in the subendothelium of blood vessels (these results were submitted as preliminary data in the grant proposal). When sections of breast cancer were examined for the presence of TSP1, we verified that capillaries were positive in some but not in all types of breast tumors.

The possibility that the TSP1 gene might be regulated by steroids has not been carefully explored, although a number of reports suggest, in an indirect manner, that this type of regulation might occur (22-24). As part of another project we have proposed to investigate this issue further

to locate steroid response elements in the TSP1 gene and identify possible trans-acting factors involved in this regulation (NIH - R29 CA65624-01). Within the context of angiogenesis, it is curious that a number of laboratories have reported inhibition of angiogenesis by steroids (25,26). It would be pertinent to evaluate whether the inhibition of blood vessel formation mediated by steroids has any correlation with the secretion of TSP1.

4. Purpose of the present work

This proposal offers a logical progression to the knowledge previously gained on the role of TSP1 in vascular biology and offers a potentially exciting avenue for the identification of a natural inhibitor of capillary growth for the treatment of human breast cancer. The successful completion of this research project will: **1.** determine whether the lack of TSP1 facilitates tumor progression and enhancement of vascular growth; **2.** identify cellular mechanism(s) by which TSP1 inhibits angiogenesis in endothelial cells; **3.** identify the receptor(s) involved in the modulation of endothelial cell behavior and examine the intracellular signaling mechanisms; **4.** determine whether TSP1 could be a selective marker for tumor-associated angiogenesis, and more importantly, **5.** determine whether the regulation of TSP1 gene can provide a natural pathway for the clinical treatment of breast cancer.

BODY

EXPERIMENTAL METHODS

A. Is the lack of TSP1 associated with growth and metastasis of malignant tumors?

Examine the progression of the vascular bed in mammary tumors of TSP1-deficient mice.

Experimental Desing/Methodology:

1. Generate mammary tumors in TSP1-deficient (tsp/tsp$^-$) mice by mating of TSP1 knock-out homozygotes with mice carrying the MMTV c-*neu* transgene

2. Analysis of the vascular bed, as well as rate of capillary extension/mm^2 of neoplastic tissue will be obtained by: a) confocal laser analysis coupled with three-dimensional reconstruction, b) determination of hemoglobin and c) endothelial cell markers. Values obtained from TSP1-deficient and from control *neu* animals will be compared.

Overall growth of the tumors and rate of metastasis will also be directly assessed and correlated with control values. Data from these experiments will concurrently provide important information on the relationship between capillary density and tumor expansion.

Determine whether exogenous TSP1 can revert/rescue the vascular phenotype of induced tumors in TSP1-deficient mice.

Experimental Desing/Methodology:

1. Slow-release pellets of TSP1 protein will be implanted in the mammary fat pads of TSP1-deficient mice carrying the MMTVc-*neu* transgene.

2. Vascular progression in tumors will be determined and compared to control-*neu* mice.

3. In addition, the localization of exogenous TSP1 protein and its half life in tumors will be assessed to gain information on the fate of exogenous TSP1 in mammary tumors.

B. What are the specific effects of TSP1 on endothelial cells engaged in angiogenesis?

Investigate the specific effect(s) of TSP1 on endothelial cells engaged in the angiogenic response.

1. Endothelial cells (EC) from normal mouse mammary gland and from mammary tumors will be isolated and characterized for their proliferation rate, secretory profile, and angiogenic potential.

2. Exogenous TSP1 will be added to EC at confluence or to cells undergoing angiogenesis *in vitro*. These experiments will be performed in both tumor-derived as well as control cells. We will determine the effect of this addition on: a) proliferation; b) migration; c) chemotaxis; and d) expression of extracellular matrix-associated molecules.

Identify the cell surface receptor(s) involved in mediating cellular responses to TSP1.

1. The presence of TSP receptors will be assessed in cultures of confluent EC, as well as in angiogenic cultures, by direct binding assays.

2. Modulation of receptor number will be analyzed after addition of TSP1.

3. Neutralizing experiments with specific anti-TSP receptor antibodies will be performed to determine which of the five recognized receptors mediates an anti-angiogenic response.

RESULTS

1. Proposed task for year 1 and 2

Task 1, Examine the progression of the vascular bed in mammary tumors of TSP1-deficient mice, Months **1-24**

a. Generate TSP1-deficient mice containing mammary tumors

b. Tumors from experimental and from control animals will be harvested and measured

c. Analyze the frame-work of capillaries in both experimental settings by morphometry

d. Analyze the frame-work of capillaries in both experimental settings using a biochemical strategy.

Task 2, Determine whether exogenous TSP1 can revert/rescue the vascular phenotype of induced tumors in TSP1-deficient mice, Months **12-30**

a. Preliminary experiments:

Determine the rate of release of [^{125}I]-TSP1 and determine its half life in mammary tumors. Adjust experimental conditions to accommodate these results.

b. Implant capsules containing TSP1 into the mammary tumors of TSP1-deficient mice

c. Determine the effect of TSP1 on the vascular density of mammary tumors as performed in Task #1 (c and d).

2. Achievements for years 1 and 2

Both tasks have been concluded at this point. We encountered several experimental problems that were solved by using alternative approaches. In several cases, the new approaches expanded the scope of our original proposal and actually yielded additional information. In addition, several related projects were explored (as indicated in Section 3), with exciting novel information to the development of vascular therapies related to the treatment of breast cancer.

Point-to-Point discussion of the proposed tasks:

Task 1, Examine the progression of the vascular bed in mammary tumors of TSP1-deficient mice, Months **1-24**

a. Generate TSP1-deficient mice containing mammary tumors

Matings proved to be more difficult than expected for two reasons: (1) TSP -/- animals had lower liters, therefore our starting population of animals was limited. In addition, the null mice had higher mortality and were less fertile than wild type animals; (2) We encountered some unexpected problems in the genotype of animals, and had to verify genetic status by Southern blot and PCR analysis to ensure results. Nevertheless, we were able to complete the task within the time-table proposed.

To obtain the F2 generation, a total of 69 heterozygous matings (tsp+/- / c-neu +) were set up. To improve the chances of matings, it was decided to induce ovulation in females. From all the matings we were able to obtain 14 c/neu + and tsp-/- females, as identified by Southern blot /PCR analysis. Eight of those animals developed mammary tumors.

A total of 18 animals with the genotype c/neu + and tsp+/+ were obtained from which 12 developed mammary tumors. In this study, we also utilized c/neu + and tsp+/- animals: 15 females were used at the same age as the other categories and all but one female developed mammary tumors.

b. Tumors from experimental and from control animals will be harvested and measured

Table I has the results from this task. We are in the process of concluding the statistical analysis of these data, although it seems from t-test evaluations, that the differences between TSP1 null animals and wild-types are statistically significant. The results indicate that in the lack of TSP1 (null mice) tumor size is greater than in its presence (wild-type) at physiological levels. Task 2 will then address if exogenous levels of TSP1 provide further benefit, reduce vascularization, and tumor size.

Although the results from this task were overall positive, we observed a great variability in the size, vascularization, and time of onset of all tumors within each experimental group. Speculations on reasons for this are two:

(1) Animal to animal variability is an issue of concern whenever animal experimentation is used and most in particular with tumor biology.

(2) Another point that deserves consideration is the possibility of redundant pathways of inhibition, even within the TSP family of proteins. Up to now, five TSP proteins have been described, none of which, with exception of TSP1 and 2 have anti-angiogenic domains. TSP-2 is not expressed in the mammary gland, therefore this was not a concern when the experimental design was originated. Further research in this area by our laboratory has now uncover two additional genes (unpublished observations) with anti-angiogenic potential. Both novel genes encode for proteins that are expressed in the mammary gland at high levels (see section 3) . The participation of these novel proteins in the regulation of vessel growth in the mammary gland along with the possible redundancy pathways is of importance and deserves further investigation.

Table I - Comparison of the weight of mammary tumors from c/neu + and tsp-/- females and controls

A. Phenotype c/neu + and tsp-/-

Mouse identification#	tumor weight (in grams)*
57675	0.72
57677	0.29
57789	0.76
57803	0.87
57912	0.25

57917	0.54
57923	0.78
58004	0.24

B. Phenotype c/neu + and tsp+/+

57630	0.47
57635	0.59
57665	0.28
57668	0.25
57702	0.35
57789	0.48
57792	0.67
57798	0.24

C. Phenotype c/neu + and tsp+/-

57310	0.37
57325	0.45
57367	0.43
57469	0.25
57494	0.47
57523	0.36
57556	0.68
57591	0.27

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*Time allowed for the development of the tumors was 8 days in all animals.

c. Analyze the frame-work of capillaries in both experimental settings by morphometry

Tumors from five animals per experimental group were analyzed to assess vascular density and vascular volume. Experiments were done using thick paraffin sections (20μm). Vessels were identified by immunocytochemistry using two antibodies: anti-vWF and PECAM-1. Sections were observed and five random areas of 100μm2 were digitized using a CCD camera connected to a Nikon epifluorescent microscope. Images were quantified using NIH 1.59Image program. Three values were obtained: pixels (intensity) of nuclei staining (as revealed by DAPI staining of nuclei); pixels from vessel staining; and estimated volume of vessel staining (this last measurement was performed using a Biorad 1600 confocal microscope). Total vessel density was assessed per area and normalized to total cell number.

Our results revealed more information than initially anticipated. A brief summary of the major findings is listed below:

(1) vessel density in TSP1 null mice was significantly higher than in wild-type controls (mean was 1.4 fold ±0.06).

(2) the overall vessel architecture was different in the null mice in comparison to the wild-type controls. While in controls the capillary networks were large, dilated, and irregular structures; the null animals showed thin capillaries with large networks (longer distance between capillaries). This finding was particularly evident after injection of the mammary artery with FITC-dextran (Task 1, d).

(3) evidence of intravascular clotting was seen in few areas in TSP1 null mice but not in control animals.

d. Analyze the frame-work of capillaries in both experimental settings using a biochemical strategy.

This task did not work as well as originally anticipated. We had proposed to use levels of hemoglobin as a determinant of vascularization. Although we were able to obtain measurements, the correlation between hemoglobin levels and capillary density did not hold true for tumors. Mostly because of hemorrhage in the interstitium and large, dilated and irregular vessels. Perhaps this technique could be effective as a measurement of blood vessel density in a non-pathological specimen. We then decided to use injection of FITC-dextran to evaluate vessel volume and vascular architecture. This procedure worked well and with the aid of a Biorad Confocal microscope, we were able to obtain information that supported and supplemented the data obtained with cross sections. The main conclusions from these studies has been listed previously (Task 1, c).

Task 2. Determine whether exogenous TSP1 can revert/rescue the vascular phenotype of induced tumors in TSP1-deficient mice, Months **12-30**

a. Determine the rate of release of [^{125}I]-TSP1 and determine its half life in mammary tumors. Adjust experimental conditions to accommodate these results.

1. Pilot studies on cleavage fragments of TSP1 protein

To estimate the concentration of TSP1 necessary for in vivo delivery of TSP1 and to have a clear idea of the functional activity of the different batches of TSP1 protein purified in our laboratory, we devised a functional assay. The assay consists on the ability of TSP1 protein to inhibit migration/invasion of endothelial cells into collagen gels containing bFGF. To suppress any endogenous protein, we used endothelial cells derived from tsp-/- mice and serum-free conditions. This assay has proven extremely useful to "normalize" our preparations of TSP1 and to guide our protein purification strategy to yield a greater percentage of active / functional TSP1.

For the execution of the assay, we have included heat-denatured TSP1, BSA alone, and protease-denatured TSP1. To our surprise, when we utilized thrombin-cleaved TSP1 the ability of the protein to inhibit migration was 10-15 times enhanced. Further, more refined experiments (thrombin-free) revealed that fragments of TSP-1, generated by thrombin proteolysis, were 35-50 times more potent than the intact protein in inhibiting migration of endothelial cells into collagen gels. These data would indicate that proteolysis is required, or at least enhances, the anti-angiogenic effect mediated by TSP1. Therefore, synthetic peptides that correspond to relevant TSP-1 subdomain(s) mimic the anti-angiogenic effect mediated by TSP1. These results provided new information on the domains of TSP1 important in the anti-angiogenic function and provided new light into the possible physiological regulation of its vascular growth suppressor activity. We are very excited about this novel findings, because they can solve problems related to delivery and metabolic processing in *in vivo* experiments. We will re-designed experiments proposed in Aim 2 to utilize thrombin-derived fragments of TSP1 (although intact TSP1 was also used).

To assess the stability of TSP1 in the tumors, the intact protein was iodinated and injected into mammary tumors. Tumors were harvested at 5min, 30min, 1h, 3h, 5h, 12h, and 24h. The experiment was performed in 5 animals that had tumors of - 0.2 to 3cm in diameter. Breakdown of the protein was seen as soon as 30 min after injection. Complete degradation was observed by 12h in the large tumors. The cleavage of TSP1 after 30 generated two major fragments: a 140kD and a 40-50kD. These fragments were also generated after treatment of the iodinated protein with thrombin and, as indicated previously, this might be an active form of the protein *in vivo*.

Clearly, TSP1 is very unstable and a continuous infusion of the protein will be necessary to maintain high levels within the tumor stroma. This finding together with further difficulties encountered in the diffusion of TSP1 from the pumps and the size of the pumps themselves made us reconsider the experimental design. An alternative approach, using stable transfected cells was used, instead of the pumps, to deliver recombinant TSP1 into the tumors. Although this approach

entailed the generation of expression constructs and stable cell lines (which significantly delayed our timetable), it allowed us to make constructs that encoded for truncated versions of the protein that will provide further information on the mechanism of action of this protein.

b. Implant capsules containing TSP1 into the mammary tumors of TSP1-deficient mice.

As discussed above, this task, as originally designed was problematic and although four large consecutive experiments were performed, the delivery of TSP1 to the tumor was not sufficient. In addition, the pumps were rather large for the animals and the amounts of TSP1 required to overcame rates of degradation would have been 100 fold higher than previously anticipated.

Our alternative approach entailed the use of stable cell lines containing TSP1 cDNA driven by the CMV promoter.

Four expression vectors were constructed:

(1) entire cDNA encoding TSP1

(2) a SacI - EcoRI fragment of TSP1 encoding for a 140kD fragment

(3) a SacI - ClaI fragment of TSP1 encoding for a 120kD fragment

(4) a construct containing the entire cDNA encoding TSP1 but containing 4 mutations in the antiangiogenic domain.

The constructs were transfected on mammary tumor cell lines (two cell lines were used: MC435 and MC 437) and selected with G418. For each construct 15 to 20 clones were isolated. The clones were evaluated for expression of the recombinant proteins by Northern and Western blot analysis. This was concluded 3 weeks ago, we are currently in the process of growing the clones to inject into nude animals.

c. Determine the effect of TSP1 on the vascular density of mammary tumors as performed in Task #1 (c and d).

This task will be performed as indicated in **c** and **d** as soon as the tumors are generated into the nude mice.

Additional activities proposed for this period

Isolation of endothelial cell strains

We have also isolated and characterized endothelial cells from tsp-/- and from tsp +/+ animals. Specifically, fifteen isolations from independent animals of each phenotype have been performed. These cells will be used for Tasks 3 and 4 and indicated in the grant proposal.

All the strains were expanded and frozen at early passage number. If time permits, we will soon (probably by January 97) initiate the experiments that have been proposed for years 3 and 4.

3. Achievements not proposed in the original grant, but related to this project

Analysis of the expression of TSP1 and vascular density during physiological expansion of the mammary gland

These experiments were initiated in year 1 of the proposed grant and partially discussed in the last progress report. All the experiments are now concluded and a manuscript describing these findings is in preparation.

Briefly, we found that TSP1 mRNA and protein are upregulated during the regression of the mammary gland associated with involution after lactation. Comparison of TSP1 null and wild-type mice revealed that the null mice are delayed in the regression of capillaries during gland involution. Analysis of apoptotic cells showed that in TSP1 null animals, the number of apoptotic cells is reduced by 23%.

Novel anti-angiogenic genes

In collaboration with Human Genome Science we have screened a number of cDNA libraries with the anti-angiogenic domain of TSP1 (referred to as the type 1 repeat). Two novel cDNAs were isolated. These cDNAs correspond to novel and independent genes that contain a 85% conserved region of the type I repeats preceded by a methalloproteinase domain. Because of the presence of the metalloprotease domain and the thrombospondin domain, these genes were named METH-1 and METH-2. The overall similarity with TSP1 is 29%, between each other, the cDNAs for METH-1 and METH-2 are 51% similar. The genes were identified in human, mouse, chicken, and xenopus by Northern blot analysis. In situ hybridization revealed high level of expression in the mammary gland, specifically in mammary epithelial cells (METH-1 and 2) and in the endothelium (only METH-2). Generation of synthetic peptides to the type I repeats region (done only for METH1 thus far) showed 56% inhibition of angiogenesis in the CAM assay. We are currently making constructs for recombinant expression of this protein and we are examining its regulation in mammary epithelial cells.

CONCLUSIONS

At the end of year two of this proposal, these are our major conclusions:

1. TSP1 null mice generate c-neu-driven mammary tumors that are statistically significantly bigger than wild-type controls.
2. Vascular density and vascular volume is bigger in TSP1 null mice than in control animals.
3. The capillary architecture in tumors present in TSP1 null animals is distinct (smaller and non-dilated) as compared to control. Furthermore the vessels of null animals present diffuse thrombosis.
4. Novel members of the TSP superfamily have been isolated. Transcripts are highly expressed in the mammary gland and at least one has shown anti-angiogenic activity in the CAM assay.

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List of Personnel involved in this project

For year 2 - 10/95 to 10/96

Dr. Luisa Iruela-Arispe - Principal Investigator - 20%
Ms. Sarah Oikemus - Research Assitant - 40%
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Ms. Sujata Guin - Undergraduate student - summer internship